Supporting Information

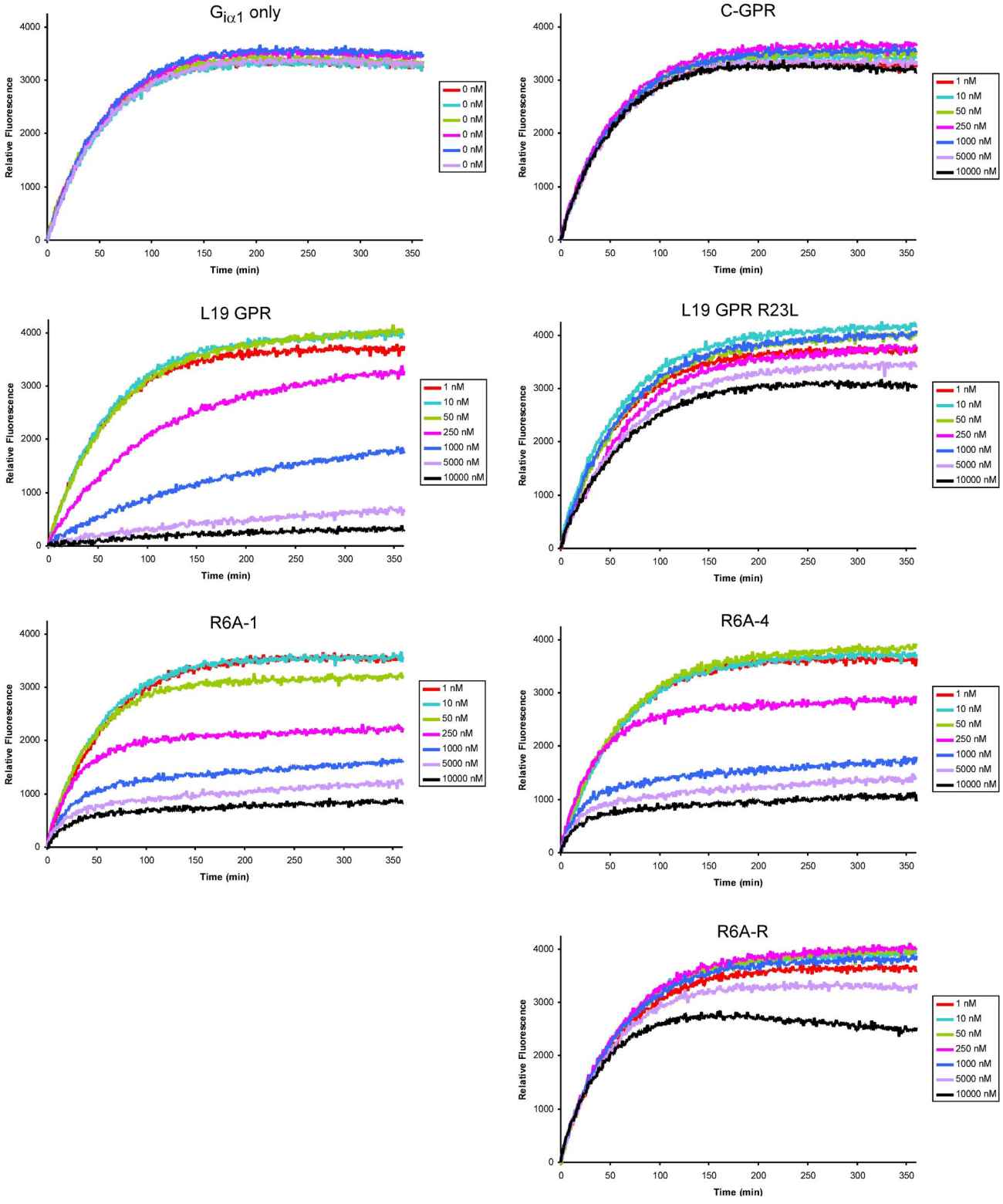
Synthesis of N,N'-D-biotinyl-2,2'-(ethylenedioxy)bis(ethylamine)-L-Cysteine

2-Cl-TrT-Cys(Mmt)-OBt resin (100 mg, 0.06 mmol capacity, Calbiochem-Novabiochem Corp., La Jolla, CA) was swelled in 3 mL of DMF at room temperature for 1 h followed by washing on a vacuum manifold with DMF and DCM. The resin was then rotated for 2.5 h at room temperature with 2,2'-(ethylenedioxy)bis(ethylamine) (500 μL, 3.42 mmol) in 3 mL of DCM. After washing as before, the resin was incubated in a solution containing D-biotin (60 mg, 0.25 mmol, dissolved in 1 mL DMSO), Pybop (130 mg, 0.25 mmol), HOBt (35 mg, 0.23 mmol), and DIPEA (90 μL , 0.52 mmol) in 2 mL of DMF. After rotating at room temperature for 9 h, the resin was washed with DMF and DCM and dried on vacuum. The resin could be stored at -20 °C until needed. For deprotection and cleavage, the resin was rotated with 5 mL of TFA/DCM/TIS (2/96/2) for 1.5 h. The cleaved biotinyl-Cys was collected by gravity filtration along with 2 additional collections using DCM. The compound was dried in vacuo, collected with MeOH, and dried again. The pellet was extracted 6 × 1 mL ether and dried in vacuo. The compound was used without further purification. ESI (MH^+) 478.2 Da (expected 478.2 Da).

Construction of pDW363A

The coding region for MBP from pDW363 was excised at the XhoI/BamHI restriction sites and purified by agarose gel electrophoresis (QIAquick gel extraction, Qiagen). The Factor Xa protease cut site was rearranged by PCR amplification of the MBP dsDNA first with primers 35.3 (5'-GGA CTA GTA AAA TCG AAG AAG GTA AAC TGG TAA TC) and 35.4 (5'-CCA TTG GAT CCT TAA TTA GTC TGC GCG TCT TTC AG) and subsequently with primers 75.1 (5'-GAG CAC TCG AGC TCT GGA GGC ATC GAG GGT CGC ATG GGT GGC ACT AGT AAA ATC GAA GAA GGT AAA CTG GTA ATC) and 29.3 (5'-CCA TTG GAT CCT TAA TTA GTC TGC GCG TC) using Herculase DNA polymerase (Stratagene, La Jolla, CA). The MBP gene was then ligated back into pDW363 at the XhoI/BamHI sites to produce the vector, pDW363A.

Supplemental Figure 1. Effect of various peptides on GTPγS binding. Fluorescence enhancement of BODIPY FL GTPγS binding to $G_{i\alpha1}$ was observed in the presence of various peptides at the indicated concentrations, as described in the Experimental Procedures. The peptide sequences are given in Table 1 of the manuscript, except for R6A-4 (SQTKRLDDQLYWWEYL). "$G_{i\alpha1}$ only" demonstrates the repeatability of the fluorescence enhancement without peptide inhibitor in 6 separate wells of a 96-well plate experiment. The differing kinetics of inhibition between the L19 GPR consensus peptide and the selected peptides (R6A-1 and R6A-4) is easily seen. The L19 GPR R23L and R6A-R mutant peptides exhibit significantly reduced GDI activity.



Supplemental Figure 1